Cardiovascular Risk Beyond Low-Density Lipoprotein Cholesterol

Manuel Mayr, MD, PhD,[a] Robert Gerszten, MD,[b,c] Stefan Kiechl, MD[d]

[a] King's British Heart Foundation Centre, King's College London, United Kingdom

[b] Division of Pulmonary, Critical Care and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

[c] Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

[d] Department of Neurology, Medical University of Innsbruck, Austria

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Correspondence:
Prof. Manuel Mayr
King's British Heart Foundation Centre
King's College London
125 Coldharbour Lane
London SE5 9NU, UK
Fax: +44 (0)20 7848 5298
Telephone: +44 (0)20 7848 5446
E-mail: manuel.mayr@kcl.ac.uk

Over the last 50 years, routine lipid parameters for risk prediction of cardiovascular disease (CVD) have not changed: clinical assays still rely on simple biochemical measurements of total triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C) is not always measured but calculated from non-HDL-C. Further, standard lipid tests quantify the cholesterol or triglyceride content of lipoproteins, without providing size-specific lipoprotein particle information. By contrast, nuclear magnetic resonance spectroscopy (NMR) provides a rapid method for distinguishing and quantifying a wide range of lipoprotein subclasses. Upon exposure to a magnetic field, distinct lipoprotein subclasses emit a unique signal that is directly proportional to their concentration. Although NMR lacks sensitivity when compared with mass spectrometry, it can be used to analyse the lipid composition of lipoprotein subclasses (1,2).

In this edition of the *Journal*, NMR-based findings from The China Kadoorie Biobank Study contribute to refining the quantitative and qualitative features of atherogenic lipid profiles (3). Very low-density lipoprotein (VLDL) particle concentrations were at least as strongly associated with myocardial infarction (MI) and ischaemic stroke (IS) as LDL particles. Further, triglycerides (TGs) were more consistently related with MI and IS across the entire spectrum of lipoprotein subfractions than cholesterol, and VLDL and remnant cholesterol outperformed LDL-C in CVD risk prediction in this Chinese population characterized with low mean LDL-C levels (85 mg/dL in the control group).

A causal role for remnant cholesterol in TG-rich lipoproteins such as VLDL has previously been suggested by meta-analysis and large-scale Mendelian Randomization studies (4,5). Similarly, mass spectrometry-based proteomics in the Bruneck Study ranked apoCIII, apoCII and apoE - three VLDL-associated apolipoproteins - as first to third with regards to CVD risk prediction (6). However, these data must be interpreted in the context of the widespread

use of statins for primary prevention in this cohort. Emerging studies suggest that cause for MI is shifting from plaque ruptures to plaque erosions, possibly due to the widespread use of LDL-C lowering therapies (7,8). With low LDL-C levels, the relative contribution of TG-rich lipoproteins to CVD risk may increase.

The China Kadoorie Biobank Study offers additional remarkable insights. First, the data contradict the conventional, yet outdated view that lipids are predominantly a risk factor for MI and are only a weak predictor for IS. In fact, the associations of lipoprotein particles with MI and IS were highly concordant, and their magnitude was only marginally lower for IS. This is supported by other studies that obtained similar findings for MI and IS, i.e. by measuring the plasma lipidome (9) or apolipoproteins like lipoprotein (a) (10-12). The most recent meta-analysis of LDL-C lowering therapies yielded a risk reduction of 24% for MI and 19% for overall stroke for more-intensive versus less-intensive therapy (13). The randomized controlled trials (RCTs) for PCSK9 inhibition and ezetimibe therapy but not cholesterylester transfer protein inhibition with anacetrapib reported benefits of lipid-lowering therapy for IS similar to or even higher than for MI (14-17). Taken together, these findings are consistent with the notion that lipids promote atherosclerosis systemically and that atherosclerosis is a main underlying cause for IS across its major subtypes. The pathogenetic relevance of lipids is obvious for IS as a downstream manifestation of atherosclerosis in extra- or intracranial large arteries, one of the main causes of stroke in China. It is also plausible for small vessel stroke. Small vessel strokes commonly arise from plaques at the orifice of the penetrating artery rather than from lipohyalinosis. The pathogenic relevance, however, may also extend to cardioembolic stroke and Embolic Stroke of Undetermined Source (ESUS). Stiffening of the aorta due to atherosclerosis and subsequent loss of the Windkessel function may elicit a diastolic backwards flow at the upper circumference of the aortic arch redirecting cardiac

emboli into the cerebral circulation. A large proportion of ESUS may actually be the consequence of fissuring of non-stenotic plaques that escape detection by carotid ultrasound. Further research into the role of lipids in the IS subtype is required to draw more definitive conclusions.

Second, the Chinese Kadoorie Biobank Study provides clarity as to one of the most controversial topics in stroke medicine - the purported protective role of lipids for intracerebral haemorrhage (ICH). In brief, none of 61 NMR parameters (lipoprotein particle concentrations and composition, particle size, and apolipoproteins) exhibited a significant relationship with ICH despite the high incidence of ICH in the Chinese population and the large number of cases (1138 ICH patients) included in the analysis. There was no signal suggesting that low cholesterol or triglyceride levels confer a higher risk of ICH as has been suggested by a large-scale meta-analysis of observational studies including 1.4 million participants and 7960 ICH cases (18). This literature-based meta-analysis may have been confounded by unrecorded comorbidities that alter lipid levels (e.g. liver and renal disease, inflammatory diseases and malignancies), inclusion of high-risk individuals for cardiovascular disease who were treated with statins, and other determinants of ICH risk (e.g. alcohol consumption and socio-economic status) that were not rigorously assessed. This view is corroborated by genetic association studies demonstrating a higher risk of ICH in carriers of variants related to high rather than low cholesterol levels (19). Moreover, the initial finding of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial that high-dose statin therapy increases the risk for ICH appears to have been an artefact of the intention-to-treat principle and was not confirmed in any other RCT testing lipid-lowering strategies, nor in subsequent meta-analyses (20,21). Even in the setting of stroke thrombolysis, there was no higher risk of ICH among statin users (22). Although it is

premature to close this discussion, the study by Holmes and colleagues in this edition of the *Journal* adds further evidence that the proposed link between low cholesterol and ICH is a misconception.

Third, now that most RCTs on HDL-C raising strategies have failed and Mendelian Randomization studies refute the causal role of HDL-C in CVD, research should target HDL composition, functionality (e.g. cholesterol efflux capacity), and other qualitative features. The Chinese Kadoorie Biobank Study suggests that the TG rather than the cholesterol content of HDL is a determinant of atherogenicity. Finally, circulating glycoprotein N-acetyl glucosamine residues - a glycan biomarker linked to inflammation and aging - showed one of the strongest associations across all three main vascular endpoints (MI, IS, and ICH). More studies are required to scrutinize these associations and to further explore cause and effect.

We are entering a new era of lipid management (12, 14-16). With a growing armamentarium of lipid-lowering therapies, patients can be more readily treated to achieve the recommended LDL-C target levels. Besides LDL-C, the therapeutic focus may broaden to tackle the residual CVD risk and include VLDL and TGs (3-6,23), as well also fatty acid composition (9, 24) and other apolipoproteins, i.e. lipoprotein (a) (10-12). It is time to advance NMR and mass spectrometry technologies for lipoprotein and apolipoprotein profiling to meet the high-throughput, low cost and standardization required for potential clinical use (25, 26). The application of multi–omics technologies might pave the way towards redefining CVD risk (27).

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